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Title page: Amplitude-integrated electroencephalography improves the identification of infants with encephalopathy for therapeutic hypothermia and predicts neurodevelopmental outcomes at 2 years of age

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Short title: aEEG is necessary to select infants for cooling

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Abbreviation list

Amplitude-integrated electroencephalography	aEEG
Basal ganglia and thalami	BGT
Bayley Scales of Infant and Toddler Development 3rd edition	BSID-III
Continuous normal voltage	CNV
Discontinuous normal voltage	DNV
Electroencephalography	EEG
Gross Motor Function Classification System	GMFCS
Hypoxic-ischemic encephalopathy	HIE
Magnetic resonance imaging	MRI
Posterior limb of the internal capsule	PLIC
Therapeutic hypothermia	TH
Time-to-normal-trace	TTNT
Severe aEEG voltage pattern (BS+LV+FT groups)	SEVP

Abstract

Objectives: To examine whether amplitude-integrated EEG (aEEG) severity-pattern as an entry criterion for therapeutic-hypothermia (TH) better selects infants with hypoxic-ischemic encephalopathy and to assess time-to-normal-trace (TTNT) for aEEG and MRI-lesion-load as 24-month outcome predictors.

Study design: Forty-seven infants fulfilling the Norwegian TH-guidelines, which do not include aEEG, were prospectively enrolled. Eight-channel EEG/aEEG was recorded from 6h till after rewarming, but only read post-discharge. Neonatal MRI brain scans were scored for summated (range 0-11) regional lesion-load. Poor outcome at 24 months was determined from Bayley Scales of infant Development 3$^{rd.}$edition as cognition and/or motor scale<85 and/or severe hearing or visual loss or death.

Results: Three aEEG severity groups were defined from the initial aEEG; continuous normal voltage (CNV;n=15), discontinuous normal voltage (DNV;n=18) and severe pattern (SEVP;n=14). CNV infants would not have qualified for TH in the CoolCap or TOBY trials. Seizure occurrence was CNV 7%, DNV 50% and SEVP 100%. SEVP infants with poor vs. good outcome had significantly longer median (IQR) TTNT: 58(9,79) vs. 18(12,19)h and higher MRI lesion-load: 10(3,10)vs. 2(1-5). Poor outcome was 3/15 CNV, 4/18 DNV and 8/14 SEVP infants. Using multiple stepwise linear regressions including only infants with abnormal aEEG (DNV&SEVP), MRI lesion-load significantly predicted cognitive and motor scores. For the SEVP group alone, TTNT was a stronger outcome-predictor than MRI-score. No variable predicted outcome in CNV infants.

Conclusions: Selection of encephalopathic infants for TH after perinatal asphyxia is better achieved by assessing the early background aEEG and including only infants with moderate or severely depressed background trace.

INTRODUCTION

Following large randomized trials of therapeutic hypothermia (TH) for term-born infants with signs of moderate or severe hypoxic-ischemic encephalopathy (HIE) (1), the International Liaison Committee on Resuscitation (ILCOR) recommended, in 2010, that TH should become standard care (2,3). Following this, there has been a significant reduction in related mortality and neurologic impairment in early childhood (3-9).

In the first 3 large randomized studies (CoolCap (10), NICHD (National Institute of Child Health and Human Development) (8), TOBY (5)), two main selection criteria for receiving TH were similar and a combination of early clinical and physiological criteria and neurological criteria describing level of consciousness and reflexes. The NICHD trial used more comprehensive neurologic criteria (8) than CoolCap and TOBY. However, CoolCap and TOBY, but not the NICHD trial, used an additional third criterion based on the degree of electroencephalographic (EEG) voltage depression assessed from the amplitude-integrated EEG (aEEG) trace. It is still debated whether selection of infants for TH needs fulfillment of all 3 criteria; physiologic, neurologic and aEEG, to indicate that the brain is affected by, or at risk of encephalopathy after moderate or severe perinatal asphyxia of likely hypoxic-ischemic origin.

The correct selection of encephalopathic infants is particularly important as the effect of cooling infants without signs of moderate or severe encephalopathy has not been tested in trials. It is not known whether cooling is beneficial, ineffective or even harmful to infants who are unwell after birth but not encephalopathic or who present in poor condition at birth but have a normal aEEG.

In order to investigate the importance of using aEEG criteria to select infants for TH we studied a cohort of infants in whom early EEG and aEEG had been obtained but was not analyzed before discharge. The aims of this study were twofold: firstly, to examine whether using aEEG data better selects infants for TH and secondly, to examine whether one or more early biomarkers e.g. time to

recovery to normal background EEG trace (time-to-normal-trace, TTNT) or brain lesion load determined from early magnetic resonance imaging (MRI) or other biochemical parameters correlate with 2-year cognitive and motor outcomes. We also compared our MRI scoring system to that used in the TOBY nested study (11).

METHODS

Study population

A single-centre (Ullevål Neonatal Intensive Care Unit, Oslo University Hospital, Oslo, Norway) selected prospectively eligible newborn term infants with perinatal asphyxia for TH between January 1st 2010 and December 31st 2011 for a cohort study. During this period, 53 newborns fulfilled the Norwegian National Guidelines for TH (12) received whole-body cooling for 3 days as standard care. In 3 infants, consent was not obtained; one had congenital myotonia, one was cooled for <72 hours and one failed the aEEG recording leaving 47(23 outborn). The Norwegian TH guidelines are based on the TOBY registry protocol (4) and use physiologic and neurologic selection criteria but not the aEEG criterion. aEEG and EEG recordings were collected from arrival in the treatment center until after rewarming and analyzed offline after patient discharge. Physiologic criteria were gestational age ≥36 weeks **AND** at least one of: a) Apgar score ≤5 at 10 minutes, b) need for respiratory support 10 minutes after birth, c) pH <7.00 or d) BE ≤-16 mmol/L in any blood sample within 60 minutes of birth. Neurologic criteria were reduced level of consciousness **and** at least one of 4 signs: 1: reduced tone, 2: abnormal eye or tendon reflexes, 3: weak or lacking sucking reflex, 4: clinical seizures. Based on this, infants were classified regarding the severity of hypoxic-ischemic encephalopathy (HIE) I, II or III (13). Four infants were retrospectively re-classified (Table 1) based on available information. Ethics approval was obtained from the Regional Committee for Medical and Health Research, South-Eastern Norway and given by the Scientific Committee of Oslo University Hospital. Written parental consent was obtained for publishing clinical and outcome data.

Clinical management

Nineteen of the 24 inborn patients and 22 of the 23 outborn infants were intubated before transport and ventilated throughout TH. The target range for $PaCO_2$ was set at 6-8 kPa during TH and hypotension was treated if the mean blood pressure fell below 40 mmHg for more than 20 minutes. All infants were sedated with morphine. Passive cooling was started as soon as perinatal asphyxia was suspected and active cooling was started in the cooling center after 155 minutes for inborn infants vs. 336 minutes for outborn infants and the mean time to reach target temperature was 176 vs. 344 minutes respectively.

The cooling system we used was a whole-body servo-controlled water-circulated jacket (CritiCool™ MTRE, Yavne, Israel) programmed to maintain the rectal temperature at 33.5°C for 72 (14). Rewarming was carried out at a rate of ≤0.5°C/hour until the rectal temperature reached 37.0°C. To prevent hyperthermia after rewarming, the cooling jacket and rectal probe were retained for at least four hours after NT was achieved (15).

EEG monitoring

All infants started EEG/aEEG monitoring around the time of starting active cooling until after rewarming (78-84h) using an 8-channel EEG (NicoletOneTMversion 5.2, Carefusion, CA, USA). Eight EEG disposable stick-on scalp electrodes (Blue Sensor BRS-50 K AmbuTM ECG electrode; Medicotest A/S, Ølstykke, Denmark) were used in a reduced montage following the International 10-20 system (16).

The expert (DO) undertaking the main off-line aEEG/EEG analyses (17) was not involved in clinical care. A single channel cross-brain aEEG trace (from central electrodes C3-P3) was derived and displayed at 6 cm/h paper speed on a semilogarithmic scale for assessment of aEEG background pattern classification. This single channel was also run as EEG to describe episodes of EEG-seizures down to a resolution of 10 second epochs. The whole 8-channel EEG recording was read for the

whole recording period for all patients (DO&JS). The aEEG background voltage and pattern was read every hour from start recording to 12h after birth. From 12h to 24h the assessments were made in 6h intervals (at 6-12, 12-18 and 18-24h) followed by 12 hourly (24-36, 26-48, 48-66, 66-72) until start rewarming when data from every second hour was recorded. The classification of the first aEEG pattern observed after starting monitoring was the "most severe pattern" seen during the first 30 minutes of recording. The aEEG traces from these 30 minutes were classified for both background voltage (18) and descriptive pattern (19,20). The infants were divided into three severity groups based on these patterns: continuous normal voltage (CNV), discontinuous normal voltage (DNV), and a combined third group named "severe voltage pattern" (SEVP) including burst suppression, low voltage or a flat trace. An electrographic seizure was defined as evolving repetitive, stereotyped waveforms with a definite onset, peak and end, lasting for at least 10 seconds of raw EEG (21,22). Status epilepticus was defined as continuous ictal activity or recurrent seizures lasting more than 50% of the EEG recording time for at least 30 minutes (21).

Moderate (DNV) or severely depressed background voltage (burst suppression, low voltage or flat trace) for at least 30 minutes within 6h (range 2-12) of birth, or non-convulsive seizure activity on any background voltage pattern (including CNV) were considered signs of encephalopathy and included for TH therapy according to CoolCap/TOBY criteria (18,20). The time taken for the aEEG to recover from a SEVP pattern to at least a DNV pattern was called TTNT. Whilst DNV is considered abnormal **before cooling** in terms of entry criteria for TH, a DNV pattern **during cooling** is not associated with poor outcome (23) hence "TTNT", can be fulfilled by either CNV or DNV **during cooling**. Anti-epileptic drugs were used for clinical seizures. The anti-epileptic drugs protocol prescribed phenobarbital 20 mg/kg as first line treatment, followed by a 2nd dose, then midazolam and lidocaine. An clinical EEG was occasionally requested and read by clinical neurophysiologists. The continuous aEEG/EEG monitoring was first read after discharge by an external collaborator (DO).

Brain MRI

Cerebral MRI was performed twice, on postnatal day 4-5 and 10-11 (24). The later scan was used for analysis, if available. Two infants who died were never scanned. Four infants (one died) only had early scans. Two independent experts (FMC, AS) assessed the MRI scans for quality, anatomy, injury pattern, hemorrhage, and evidence of venous sinus thrombosis (24). The Rutherford scoring system was used (11) with separate scores for basal ganglia and thalami (BGT), white matter and cortex on a scale of increasing injury severity of 0-3, and for the posterior limb of the internal capsule (PLIC) on a scale of 0-2. A total injury score was calculated giving a range from 0 (no lesions) to 11 (maximum lesion load*).* We also applied the binary MRI-score for poor and good outcome to our dataset developed for the nested TOBY study (20).

Other measurements

Serial plasma glucose, lactate (the duration in hours after birth before the plasma lactate decreased to ≤5mmol/L), lactate dehydrogenase at 72h (25), serum cardiac troponin T, CPR and adrenalin during resuscitation and seizure variables were used in the exploratory analysis.

Outcome assessments at 2 years

A clinical neuropsychologist (GL) undertook the Bayley Scales of infant Development, 3rd edition (BSID-III) (26) and a pediatric neurologist (ES) the neurologic examination (27) at 2 years; neither was involved in the neonatal care and both were blinded to the MRI and EEG results. Eight parents did not speak a Scandinavian language and no interpreter was used. Because this might affect the language scores, only cognitive and motor data were included in the final analysis (28). The Gross Motor Functional Classification Score (GMFCS) was determined for those children with cerebral palsy. GMFCS levels 3-5 were classified as poor outcome and <3 as good motor outcome. Cognitive and motor development was considered in the normal range if the composite BSID-III score was ≥85 (29;30), moderate delay was defined between ≤2 SD and >1SD below normal (70-84) for either cognitive or motor scores and severe delay was cognitive or motor scores <70. For binary analysis, good outcome included both cognitive and motor scores ≥85 without vision or hearing impairment.

Poor outcome was defined as death or one or more of cognitive or motor scores <85, a GMFCS of 3-5, or severe vision or hearing impairment. In some analyses, we allocated infants who died a value one unit lower than the lowest a survivor could get on the BSID-III (55 for cognition scale score) e.g. 54. The corresponding lowest value for BSID-III motor score is 45 for a survivor, so 44 was used for the infant with MRI who died.

Statistical analysis

Statistical analyses were performed using SPSS 23 (SPSS, Chicago, IL, USA). A p-value <0.05 was considered statistically significant. Demographic and clinical data were summarized at baseline as median (IQR). Two group comparisons were undertaken with either the Mann Whitney Wilcoxon or Kolmogorov-Smirnov test (which was used when the two groups had very different distributions). Tables of 2x2 were analyzed with the 'N-1' chi-squared test (31). Those methods are robust and suitable for use on small datasets where there is limited knowledge about the distributions. Multiple stepwise linear regression analysis was performed to explore the ability of early biomarkers to predict BSID-III cognition or motor outcome at 2 years. We allowed the following independent variables to participate in the analysis (table 2): birth weight, number of antiepileptic drugs used, time (hours) for plasma lactate to decrease to ≤5 mmol/L, number of hours with inotropic support, lactate dehydrogenase levels at 72h, MRI lesion load and TTNT(only applicable for the SEVP group). The distributions of the residuals were always inspected for outliers, which were not found. The factors used for correct recruitment to TH (pH, BE, Apgar score, need for ventilator support at 10 minutes after birth, HIE grading and aEEG pattern at 6h) were not used in the regression. Infants who never regained a normal trace after rewarming were allocated 78h in the regression analysis.

RESULTS

Table 1 shows the demographic, baseline, clinical and outcome variables for the 47 included infants divided into three severity groups based on their initial aEEG pattern. Fifteen infants had CNV

(normal), 18 had DNV (moderately abnormal) and 14 had SEVP (severely abnormal) patterns. The median start time of aEEG monitoring for the whole cohort was 6h, the cut off-time for starting TH in the trials. The aEEG was however applied earlier in the CNV than the DNV and SEVP groups, as more CNV were inborn (median 4h; inborn/outborn ratio 11/4 vs. DNV 7h; 7/11 and SEPV 6h; 5/9).

The 15 infants with CNV did not meet the criteria used for entry to the CoolCap and TOBY randomised trials. Thus, 32 infants had moderate or severely abnormal aEEG background voltage (18) 18 with DNV and 14 with SEVP patterns (19).

HIE grading (as in the TOBY trial)

Of the 47 included in the study, 11 were severe (HIE III) of whom 8 had a poor outcome, 32 were moderate (HIE II) of whom 7 had a poor outcome and 4 were mild (HIE I), all with a good outcome.

Neurodevelopmental outcome at 2 years

Forty-one infants were seen at a median age of 24 (22-28) months and their neurodevelopmental outcomes are presented in Table 1. Overall 15 infants had a poor outcome including three who died. Three infants developed CP, two with a quadriplegic pattern (GMFCS 4 or 5) and both with BSID-III scores <70. One of these 2 infants needed bilateral hearing aids and none in the whole cohort was visually impaired. One infant (DNV group) had a hemiplegic pattern (GMFCS 1) with BSID-III scores >84 and classified as having a good outcome. Only one child, without cerebral palsy but with BSID-III scores <85, (DNV group) had seizures requiring regular anti-epileptic drugs at 2 years. Outcome details for individual surviving children with poor outcome in the three aEEG groups are given in Table 1.

Differences between the CNV, DNV and SEVP groups

There were no significant differences between the CNV, DNV or SEVP groups in physiologic criteria except that significantly more SEVP infants received cardiopulmonary resuscitation including adrenalin compared to CNV and DNV infants. Clinical variables assumed to be related to organ failure

rather than encephalopathy were not different between CNV and DNV infants except that there was no infants with a supplementary oxygen requirement in the CNV group and the serum cardiac troponin T was higher in the SEVP than in the DNV group.

The occurrence of any seizures (both clinical and electroencephalographic) was significantly lower in the CNV than the DNV group (7% vs. 50%). The SEVP group clearly showed more signs of abnormal brain function with longer median TTNT (18.5h), a larger proportion of infants with any seizures compared to DNV (100% vs. 50%) and greater MRI lesion-load (median MRI score of 2.5 (range 0-11) vs. 1 (range 0-6). Cognitive and/or motor BSID-III scores were between 70 and 84 in 3/15 CNV and 4/18 DNV infants.

Relation between biochemical, aEEG and MRI biomarkers and outcomes in infants with abnormal aEEG

A series of variables were entered using a stepwise linear regression to investigate an association with cognition and motor scores (table 2). MRI (0-11) lesion-load showed a strong negative association with cognition (p=0.000); for each point increase in MRI lesion load score, cognition score fell by 3.38 points. TTNT can only be analysed in infants who start with a poor trace (SEVP) and TTNT showed a strong negative association with both cognition and motor scores in this group. TTNT was significantly longer in SEVP infants with poor outcome 58.5h vs. 18h (p=0.03) for good outcome.

The correlation between the cognition score at 2 years and the MRI score for the infants who had either a DNV or SEVP aEEG pattern 6h after birth is displayed in figure 1a. There is a strong linear relationship; r^2=0.35 for DNV and r^2=0.72 for SEVP. Infants with a DNV pattern had an MRI lesion load ranging from 0-6, and those with SEVP from 2-11. Figure 1b shows the corresponding data for the CNV group only, showing no relationship between cognition score at 2 years and the MRI score r^2= 0.06. The motor score fell by 6.41 BSID-III points for every 10h increased duration in TTNT in the SEVP group, table 2. Figure 1c shows the relationship between TTNT and BSID-III motor score in the SEVP group, the longer the TTNT the lower the motor score.

Comparison with TOBY using the scoring system used in the nested sub study

In the TOBY nested sub-study (11), 131 of 325 infants undergoing TH or standard care at normothermia had MRI scans examined and a binary MRI scoring system predicting poor outcome at 18 months was proposed if there was at least one of the following 4 regional MRI severity scores: BGT=2 , BGT= 3, PLIC=2 or white matter=3. When applied to the group with aEEG abnormalities in our study, the nested TOBY scoring only predicted 4 of 8 infants correctly to have a poor outcome and 14 of 20 infants to have a good outcome. Thus the positive predictive value for poor outcome was 50% (4 of 8) the negative predictive value for poor outcome was 70% (14 of 20), specificity of 78% (4 of 18) and sensitivity of 40% (4 of 10).

Seizures and outcome

Twenty-four of the 47 infants had both clinical or electroencephalographic seizures between birth and the end of rewarming; 1 was in the CNV group, 9 in the DNV group, and 14 in the SEVP group (Table 1). All infants with clinical seizures received anti-epileptic medication. Of the 14 with clinical seizures only, 11 occurred before aEEG monitoring started and in the remaining 3, the seizures were not verified on offline EEG. Ten infants (7 with a clinical correlate) had electroencephalographic seizures diagnosed offline, which were classified as single, repetitive or status epilepticus. Of the 6 infants with status epilepticus, 3 had a good outcome (one from the DNV group and 2 from the SEVP group) and 3 had a poor outcome (all from the SEVP group). The median duration of TTNT was 19h for the good and 75h for the poor outcome groups; the median duration of status epilepticus however was similar, 5h and 6h respectively, regarding outcome. Having any seizure or not, when entered into the stepwise regression, did not improve outcome prediction.

DISCUSSION

The first three large randomized controlled trials of TH (5,8,10) included infants with moderate or severe, but not mild encephalopathy. These trials used similar entry criteria except for one

examination, the presence of a depressed aEEG as a marker of encephalopathy, which was not in the NICHD entry criteria. Now, in 2016, it is apparent that centers outside the US, in particular those who include infants for TH without aEEG, do not use the strict neurologic criteria developed for the NICHD cooling protocol, but apply the less strict neurologic criteria developed for CoolCap and TOBY. We suggest that this practice is likely to over-recruit infants to TH as indicated by the 15 infants with CNV in this study who did not meet the CoolCap/TOBY aEEG criteria but were correctly included according to the Norwegian guidelines. This means that one in three infants in our cohort did not have an abnormal aEEG that would have qualified for TH in the CoolCap/TOBY trials. In our study, there were no differences in the physiologic criteria between the CNV and DNV groups. However only the DNV and SEVP groups, not CNV had clinical findings associated with measures of abnormal brain function: seizure burden, increased MRI lesion load and prolonged TTNT (for SEVP only).

We have previously published comparable aEEG data from the UK for both cooled and non-cooled infants (23). In this UK cohort, 43 infants were treated with TH and 31 with normothermia using the CoolCap/TOBY protocol including the aEEG entry criterion. Experienced investigators, who introduced the aEEG-prediction method to neonatology, read the traces retrospectively (19,20). As in our current study some infants (8/43 (18%)) were cooled despite having a normal aEEG pattern at entry on retrospective analysis. The outcome severity is different between this former UK and the current Norwegian cohorts; 41% poor outcome (23% mortality) in the UK cohort and 30% poor outcome (6.3% mortality) in Oslo. This difference is best explained by different distributions of aEEG severity at entry; UK had 37% of infants with CNV or DNV patterns combined compared to Oslo with 70%. In the SEVP group comprising burst suppression, low voltage and flat traces, however, the relative morbidity/mortality was very similar; there were 27 such infants in the UK study with 10 good survivors, 7 poor survivors and 10 deaths and in our current study, 14 infants had SEVP with 6 good survivors, 5 poor survivors and 3 deaths. If one applies aEEG pattern at entry as standard patient classification for TH one could easily and correctly compare outcome between different studies because those with normal, moderate or severe patterns to start with could be identified.

Our second aim was to examine whether data from the aEEG readings of TTNT correlated with outcome at 2 years assessed using the BSID-III. We used cognitive and motor scores as separate outcome markers in the 32 infants with abnormal aEEG. Using multiple stepwise linear regressions for these patients, MRI lesion load was the only factor that correlated significantly with the cognition and motor score, table 2. For the SEVP group, TTNT was the best predictor for cognition and motor scores, table 2. TTNT has also the advantage of being determined from an early bedside examination giving robust information during the TH period.

When running the same multiple stepwise regressions on the CNV group only, no variable predicted outcome. Both aEEG and EEG are useful tools for assessing seizures during TH. It is documented that the incidence of neonatal seizures has not changed in the cooling era, but overall seizure burden has been reduced. Neonatal seizures are often non-convulsive (32,33). Using a fetal sheep model of neonatal HIE, Alistair Gunn's group has suggested that seizures occurring during TH do not injure the brain to the same extent as seizures during normothermia (34). This, in addition to our small and mild cohort may be the reason why we did not find a different degree of injury in those that had evidence of any seizures compared to those that did not.

However if TTNT or MRI were not in the regression, the duration of status epilepticus was the strongest outcome predictor in the SEVP group.

The predictive value for outcome of cerebral MRI is thought not be affected by TH (35). The positive predictive value for poor outcome in a nested sub-study of the TOBY trial was 76 and 74% in the cooled and non-cooled groups respectively (11). In our cohort, however, the TOBY scoring system did not predict binary poor outcome, the PPV being 50%. No other prospective study has tested and published the nested TOBY Rutherford scoring system. One of the reasons for the discrepancy may be that the overall injury is milder in our study, even after exclusion of the 15 infants with a normal aEEG at entry. Other scoring systems of MRI severity within the first two weeks after birth have predicted outcome at 1-3 years in both cooled and non-cooled infants (11,36-38). However, as with

the TOBY scoring system used here, these other protocols need to be verified in clinical cohorts unrelated to the infants in whom the scores were developed.

There are limitations to this small cohort study, particularly that there were relatively few infants with severe injury. We did not use the language domain of the BSID-III examination as several children came from families where neither parent spoke Norwegian. This approach is similar to comparable outcome studies (30;39). Additionally, clinical neurology scoring at entry which grades the level of encephalopathy into mild, moderate or severe showed that 4 infants with mild HIE who were entered into the study all had a normal outcome.

In this cohort of infants treated with TH, early MRI pathology correlated well with 2-year cognitive outcome, and for the SEVP group, the time it took for the aEEG to regain a normal background voltage (TTNT) correlated with motor outcome. Additionally, previously reported MRI criteria did not predict poor outcome, which suggests that MRI scores developed in one study may not directly translate to other infant cohorts.

A lot has changed since the first hypothermia trials were published when all infants were warmed to 37°C within 30min of birth and cooling was only first started by 4-5h of age. Today, we resuscitate with room air, passive cooling is started early and hypocapnia, hypoglycemia and stress are minimized. The combination of these factors, not only TH, may contribute to reduced rates of morbidity and mortality. Another consideration is possible harm from subjecting a newborn who would not have qualified for TH in the trials to 3 days of intensive care and its risky procedures.

Our data are an important reminder that infants who are recruited for TH should show signs of encephalopathy. As the effects of cooling in the absence of overt encephalopathy are not known, aEEG depression is an important selection requirement in addition to the CoolCap and TOBY entry criteria in order to prevent over-treatment and ensure that only those infants who are suitable for TH are cooled.

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None had any conflicts of interest.

Table and Figure legends

Table 1 The table presents early clinical and 2-year outcome data from the 47 infants divided into 3 groups according to their aEEG pattern recorded at around 6 hours after birth. The aEEG and EEG were read retrospectively after discharge. Fifteen infants presented with a CNV aEEG pattern, 18 with DNV and 14 with SEVP. Column 4 gives statistical comparisons between the CNV and DNV groups and column 5 between the DNV and SEVP groups when applicable. TTNT can only be assessed for those starting with SEVP, not CNV or DNV.

Table 2 Five or six variables were entered using a stepwise linear regression to investigate an association with the cognitive and motor BSID-III scores in infants with an abnormal aEEG (DNV and or SEVP) or without aEEG abnormalities (CNV).

Figure 1a shows the cognition score versus MRI lesion-load for included infants with aEEG abnormalities; DNV (blue circles) and SEVP (red triangles). Data were available for 27 of 32 (2 died without MRI, 3 missing BSID-III). The figure shows the line of regression +/- 1SD. No DNV infant had a lesion load >6, and 14 of 18 had a good outcome. Infants with an initial SEVP pattern had an MRI lesion load range from 2-11 and 8 of the 12 infants presented in the figure had a poor outcome.
Figure 1b shows the corresponding graph for the infants with CNV; blue squares. There was no relation between cognition and MRI score.
Figure 1c shows a strong correlation between motor score and time-to-normal trace (TTNT) for SEVP infants.

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